2389-Pos Board B526
A Langevin Dynamics Algorithm for Coarse-Grain Modeling of Protein Clusters
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A variety of recent experimental results have highlighted the importance of spatial localization and clustering in cellular signaling pathways. We have called such non-stoichiometric dynamic clusters "pleiomorphic ensembles" (PE). The participation of PEs often significantly changes the dynamic and equilibrium properties of signaling networks, but the mechanisms behind such changes can be unclear, and computational modeling of these effects is hampered by the lack of efficient and appropriate methods to address such questions. Non-spatial models can reproduce some features of clustering, such as phase transitions, but cannot capture features such as steric hindrance and PE diffusion, which may have important biological consequences. Publicly available spatial stochastic solvers, such as Smoldyn or MCELL, treat all particles as points and thus cannot model spatial organization and excluded volume. In principle, clustering could be modeled with molecular dynamics simulations, but typical PE sizes and time scales preclude such models with current computational hardware.
To overcome these limitations we have designed a general purpose Langevin dynamics simulator that models proteins as a set of sites connected by stiff links. The sites are modeled as impenetrable spheres, which captures the effects of excluded volume and steric hindrance. The program allows for zero order creation reactions, a variety of first order reactions such as dissociation reactions and transitions between states of a given site (e.g., active or inactive), and second order binding reactions between sites.
We apply our program to study cluster formation in the nephrin-Nck-N-wasp system, which is important for kidney podocyte function. We compare our results to the previously published analysis of this system based on a non-spatial simulator (Falkenberg et al. Biophys. J. 2013), to explore the consequences of steric crowding and diffusion on cluster dynamics. (Supported by NIH grants TRO1DK087650 and P41GM103313)

2390-Pos Board B527
DFGmodel: Predicting Protein Kinase Structures in Inactive States for Structure-Based Discovery of Type-II Inhibitors
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Protein kinases exist in equilibrium of active and inactive states, in which the aspartate-phenylalanine-glycine motif in the catalytic domain undergoes conformational changes that are required for function. Drugs targeting protein kinases can bind the primary ATP-binding site of an active state (type-I inhibitors) or utilize an allosteric pocket adjacent to the ATP-binding site in the inactive state (type-II inhibitors). Limited crystallographic data of protein kinases in inactive state hampers the application of rational drug discovery methods for developing type-II inhibitors. Here, we present a computational approach to generate structural models of protein kinases in the inactive conformation. We first perform a comprehensive analysis of all protein kinase structures deposited in the Protein Data Bank. We then develop DFGmodel, a method that takes either a known structure of a kinase in active conformation or a sequence of a kinase without a structure, to generate kinase models in inactive conformation. Evaluation of DFGmodel's performance using various measures indicates that the inactive kinase models are accurate, exhibiting RMSD of 1.5 Å or lower. The kinase models also accurately distinguish type-II kinase inhibitors from likely non-binders (AUC $>$ 0.70), suggesting that they are useful for the utility of virtual screening. Finally, we demonstrate the applicability of our approach with three case studies. For example, the models are able to capture inhibitors with unintended off-target activity. Our computational approach provides structural framework for chemical biologists to characterize kinases in the inactive state and explore new chemical spaces with structure-based drug design.

2391-Pos Board B528
Virtual Reality Environment for Patching and Imaging in Brain Slices
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We are developing a program that constructs a 3D representation of a virtual workspace that visualizes the brain slice and the perfusion chamber, the patch-clamp and other recording electrodes, and the imaging objectives. The user can control the operation and 3D positioning of the instruments relative to the sample, with real-time visual feedback. Specific cells can be bookmarked and linked to optical and electrical recordings, and the 3D workspace can be saved for later viewing and data analysis. We are using this software platform to explore ion channel properties and their contributions to cellular and network interactions in brain slices. This unified interface provides a more streamlined approach for combining electrophysiology with structural and functional imaging.

Optical Microscopy and Super-Resolution Imaging II

2392-Pos Board B529
3 Color - 3 Dimensional STED Nanoscopy
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The stimulated emission depletion microscopy (STED) is a super-resolution technique that enables to overcome the diffraction barrier, allowing to distinguish details of cellular as well as molecular structures, not visible with a conventional confocal microscope [1].
Here we present the development of a custom made versatile STED microscope, based on a super-continuum pulsed laser source endowed with 2 high power STED laser beams, at 715 and 745 nm, respectively [2]. In order to obtain three-dimensional super-resolved images, we split the STED beam into two parts. One of the beams passes through a vortex phase plate which creates a donut shape beam for lateral resolution enhancement. For the other beam we introduced a home-made phase plate which introduces a phase delay of π in the center, creating a z-donut to increase resolution in the axial direction [3]. We can tune the microscope PSF adjusting the ratio of the power in the two depletion pathways, to achieve, for example, an isotropic resolution. Additionally in order to perform three color imaging we paid particular attention to chromatic aberrations and we optimized the setup accordingly.
Therefore by using proper fluorophores, we are able to do 3 color super-resolved imaging.
Thanks to this completely custom made optical architecture, designed for fast scanning, 3D and three color acquisition, we are able to perform multimodal live cell imaging.
The achieved 3D resolution discloses morphometric properties at the nanoscale which are completely hidden to the confocal observation.
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2393-Pos Board B530
Efficient Integrated 3D and Multi-Color Single Molecule Super-Resolution Imaging
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PALM, STORM, and related super-resolution localization microscopies were initially limited to a 2D resolution improvement in a single color. Since then a number of methods for both 3D localization and multi-color imaging have become well established. A particularly promising approach to multi-color is ratiometric imaging, which allows spectrally similar fluorophores to be distinguished and imaged simultaneously, minimizing the acquisition time as well as artifacts due to drift, sample motion, and chromatic aberration. By splitting each single molecule signal across 2 channels, however, ratiometric multi-color incurs a small penalty in signal to noise (SNR) and contrast. 3D methods either work by aberating the PSF or by splitting the image, also incurring an SNR penalty. Added to this are insertion losses from the imperfect optical systems used.
To date, the combination of 3D and multi-color has typically relied on 'chaining' a 3D method and a color method - e.g. using an image splitter for ratiometric color together with an astigmatic lens for 3D. This incurs an SNR 'double-hit', making it challenging to routinely obtain high quality multi-color 3D images. By devising a system from scratch to estimate both color and 3D position, much of the redundancy in the 'chaining' approach can be eliminated and the SNR hit minimized.
In this presentation we discuss our experience with two such integrated approaches - the use of a focal offset within a spectral splitting device to allow simultaneous biplane 3D and ratiometric color with only two images on the

camera, and the adaptation of the phase-ramp 3D method to encode fluorophore color in the relative intensity of the two PSF lobes.

2394-Pos Board B531

Three-Dimensional Super-Resolution Protein Localization Correlated with Vitrified Cellular Context

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We demonstrate a cryogenic super-resolution correlative light and electron microscopy of vitrified specimens which were prepared by high pressure freezing and cryo-sectioning to maintain a close-to-native state with better fluorescence preservation. Several fluorescent proteins were found photo-switchable and emitted much more photons under cryo-condition, hence resulting in higher localization precision. We observed nice correlation of a mitochondria protein with mitochondria outer membrane at nanometer resolution in three dimensional.

2395-Pos Board B532

3D Microscopy of Rod-Shaped Bacteria Reveals Roles of MreB in Diameter Control and Center-Line Curvature

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Over the past few years, our lab has developed a method for precisely determining the shape of bacterial cells in 3D by fluorescence microscopy. We use this method to measure the position of MreB in relation to the cell surface. To ensure that our measurements of fluorescent MreB reflect untagged MreB as nearly as possible, we have integrated our fusion at the native locus. This construct has unperturbed mass doubling times and proper rod-like shape. The shape and localization measurements that we report here are measured as snapshots from hundreds to a few thousand cells per condition.

MreB, a membrane-binding structural homolog of actin, is one of the key players in properly patterning growth of the bacterial cell wall and thereby the shape of the cell. In one series of experiments, we show that the helical pitch of MreB filaments in E. coli is highly correlated to the diameter of the cell. This correlation holds for E. coli whose diameter has been altered by treatment with sub-lethal doses of the MreB targeting drug A22 and for single point mutants in MreB.

Additionally, MreB polymers show a clear preference for regions of negative Gaussian curvature. We hypothesize that cells use this geometric sensing mechanism to straighten their centerlines [Ursell et al., PNAS 2014]. When this curvature preference is abolished (E. coli MreB A158T), cells are more frequently branched. In the smaller, comma shaped bacterium Caulobacter crescentus, the curvature enrichment profile shows a plateau region at low positive Gaussian curvature. This plateau enables Caulobacter cells to grow with their characteristic comma shape instead of as straight rods.

2396-Pos Board B533

Enabling Single-Molecule Detection in Living Cells: Ultra-Sensitive Microscopy and Spectroscopy in 3D

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Single-molecule approaches can now allow us to follow the movement, interactions and conformational dynamics of individual molecules in real-time, thus providing novel insights in complex biochemical systems that have remained masked in the ensemble averaging of traditional bulk biochemical approaches. However, most single-molecule experiments are based on in vitro reconstituted systems with either surface-immobilized or freely-diffusing biomolecules in dilute conditions. Observing individual biomolecules in their native, crowded intracellular environment currently remains an extremely challenging task.

Pushing the limits of spatial/temporal resolution, and more importantly detection sensitivity, to enable performing single-molecule assays using fluorescently labeled biomolecules inside living cells would open many new frontiers in biochemical investigations; however few generally applicable viable approaches have been described. Here we show the theoretical basis and experimental validation of a new approach based on spatially-targeted manipulation of the fluorescence capabilities of single individual fluorophores. We show that with appropriately shaped on-off switching light-fields as well as by carefully considering the time-evolution of the fluorescent state at different xyz positions, sharper single-molecule images can be obtained. Furthermore, we demonstrate increased sensitivity in real-time detection of inter-molecular associations of individual target-probe molecular partners. Our results additionally indicate the potential for increased sensitivity using our approach to detect individual intracellular factors, such as the components of the RNA Polymerase II transcription machinery in the nucleus of single-living cells.

2397-Pos Board B534

Analysis of Nanoscale Protein Clustering with Quantitative Localization Microscopy

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Dynamic clustering of proteins on the nanoscale is a vital step in many signaling processes and other cellular functions. Localization microscopy techniques such as PALM and dSTORM provide methods to localize complexes to nanoscale resolution. Quantification of the number of underlying protein subunits is more difficult, however, due to the complex photophysics of the fluorophore labels. Re-activation, stochastic blinking, and incomplete detection all contribute to over- and under-counting artifacts.

In order to generate an accurate quantification of protein subunit numbers we have developed a method based on fluorophore blinking kinetics captured in PALM microscopy. This approach takes advantage of both spatial and temporal information to form adaptive discrimination criteria and avoids both over- and under-counting quantification errors. The technique presented can correctly identify and quantify molecular clusters in simulated data with 98% accuracy. We will present additional data on the application of this technique in quantifying the nature of clusters of proteins involved in the immune synapse and immune signaling.

2398-Pos Board B535

Video-Rate Super Resolution Microscopy in Living Cells

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Super resolution microscopy based on single-molecule localization relies on precise and accurate localization of large numbers of single-molecules. However, the necessity of accumulating large numbers of localization estimates limits the time resolution typically to seconds to minutes[1,2].

Two major limitations are the acquisition speed and the photon budget. Replacing the usually used EMCCD with a recently introduced sCMOS camera results in leaps in both acquisition speed and effective quantum efficiency. However, the intrinsic pixel-dependent Gaussian noise of the sCMOS cameras introduces localization artifacts and greatly reduces the reliability of the results.

Here, we present a set of specially designed methods that characterize an sCMOS camera for the first time and allow for unbiased and precise localization analysis. Using this method we demonstrate Cramer-Rao lower bound-limited single-molecule localization with an sCMOS camera. Combining the novel algorithm with a recently developed multi-emitter fitting algorithm[3], We shortens the typical acquisition time for fixed samples by up to two orders of magnitude without compromising the field of view. Furthermore, we demonstrate localization-based super-resolution microscopy in live cells by monitoring dynamics of protein clusters, vesicles and organelles at a temporal resolution from 2 to 30 frames per second[4].

These methods allowed us to investigate cytokinetic apparatus in live fission yeast at 20-30 nm resolution. In general, the significantly improved temporal resolution allows super resolution imaging of a large range of dynamic events in living cells.

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2399-Pos Board B536

Dual-Objective Pointillism Microscopy Setup with Interferometric and Astigmatic Detection

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In recent years, under the general term of pointillism microscopy many techniques have been developed which employ sequential imaging of photoswitching fluorophores to circumvent the diffraction barrier in light microscopy